



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

*November 18, 2004*

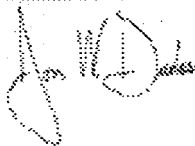
**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.**

**APPLICATION NUMBER: 60/514,678**

**FILING DATE: *October 27, 2003***

**RELATED PCT APPLICATION NUMBER: *PCT/US04/35586***

Certified by



Jon W Dudas

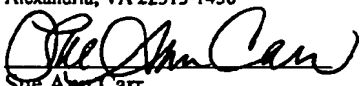
Acting Under Secretary of Commerce  
for Intellectual Property  
and Acting Director of the U.S.  
Patent and Trademark Office



**U.S. DEPARTMENT OF COMMERCE**  
**PATENT AND TRADEMARK OFFICE**

**PROVISIONAL APPLICATION FOR PATENT  
COVER SHEET**

Address to: Mail Stop Provisional Application  
Commissioner for Patents  
PO Box 1450  
Alexandria, VA 22313-1450  
(703) 308-4357

Certificate Under 37 CFR 1.10  
Date of Deposit: October 27, 2003  
I hereby certify that this correspondence is being deposited with the United States Postal Service as "Express Mail" service under 37 CFR 1.10 on the date indicated above addressed to Mail Stop Provisional Application, Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450  
  
Sue Ann Carr  
Express Mail No. ER 319 461 943 US

22154 U.S. PTO  
60/514678



102703

This is a request for filing a Provisional Application for Patent under 37 CFR 1.53(c)

Inventor(s) and Residence(s) (city and either state or foreign country):

Last Name	First Name	Middle Initial	City	State or Country
Gorczynski	Michael	J.	Charlottesville	Virginia
Bushweller	John	H.	Charlottesville	Virginia
Brown	Milton	L.	Charlottesville	Virginia

Title: **SYNTHESIS AND EVALUATION OF SUBSTITUTED 4-ARYLOXY AND 4-ARYLSULFANYL-PHENYL-2-AMINOTHIAZOLES AS INHIBITORS OF HUMAN BREAST CANCER CELL PROLIFERATION**

20 Sheets of specification.  
       Sheets of drawings.

University of Virginia Patent Foundation claims small entity status as a nonprofit organization (37 CFR §§1.27(a)(3) and (c)). The Commissioner is hereby authorized to charge the Small Entity Fee of **\$80** to Deposit Account No. 50-0423.

Please direct all communication relating to this application to:

John P. Breen, Esq.  
Patent Counsel  
University of Virginia Patent Foundation  
1224 West Main Street, Suite 1-110  
Charlottesville, VA 22903 U.S.A.


Customer No. 34444  
Telephone: (434) 243-6103  
Fax: (434) 924-2493

This invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. The government has certain rights in the invention.

YES ☐ NO ☒ Grant No. \_\_\_\_\_

Dated: October 27, 2003

Respectfully submitted,

By:   
John P. Breen (Reg. No. 38,833)

## **Synthesis and Evaluation of Substituted 4-Aryloxy-and 4-Arylsulfanyl-phenyl-2-aminothiazoles as Inhibitors of Human Breast Cancer Cell Proliferation**

### **Background**

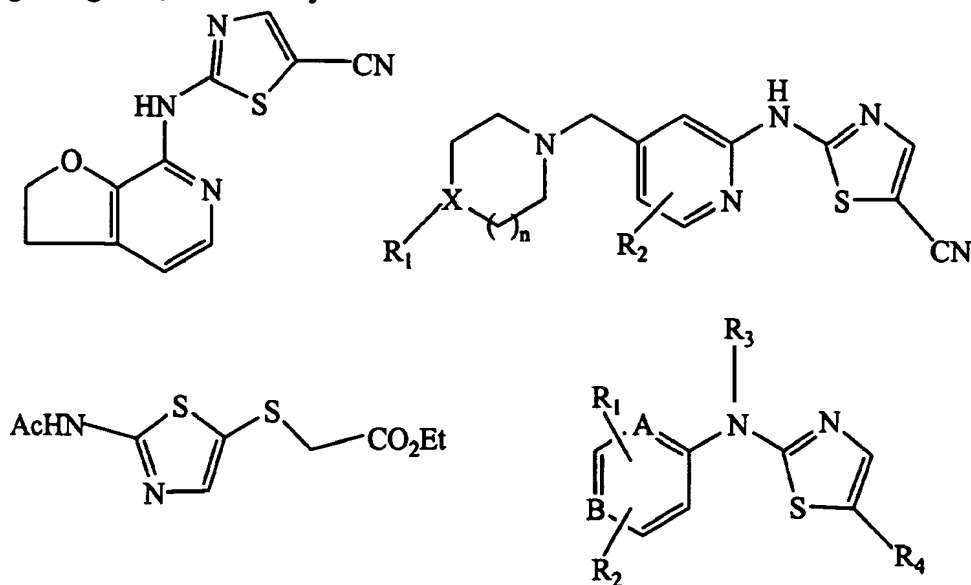
Breast cancer is the most common cancer and second most cause of cancer death among women. The National Cancer Institute (NCI) estimates 211,000 new breast cancer cases and 40,000 deaths for 2003. There are two types of breast cancer: in situ and invasive. In situ breast cancer is divided into two subtypes: ductal cystic (DCIS) and lobular cystic (LCIS). Infiltrating ductal carcinoma is the most common type of invasive breast cancer accounting for approximately 70% of all breast cancer diagnoses. Another type of invasive breast cancer is infiltrating lobular carcinoma. This form accounts for 5-10% of invasive breast cancers. In addition, there are a few less common histologic subtypes of invasive cancer including medullary, mucinous, and tubular.

Current treatments for breast cancer depend on the patient's type of breast cancer. Local incision plus radiation or a simple mastectomy are possible treatment options for patients with DCIS. There is a 20-30% risk of developing invasive breast cancer for women with LCIS and as a result, it is managed with careful bilateral breast observation. Invasive cancers are treated surgically by either a modified radical mastectomy with axillary lymph node dissection or a lumpectomy with axillary lymph node dissection followed by local radiation. Surgical treatment options are standard treatment therapies for DCIS and invasive cancers followed by post-surgical (adjuvant) treatment. These adjuvant therapies have improved survival rates of women with these types of breast cancer. In addition to surgical options, adjuvant drug therapy can decrease the risk of systemic recurrence by approximately one third.

Adjuvant treatments include cytotoxic chemotherapy (i.e. paclitaxel, doxorubicin), hormonal therapy (i.e. tamoxifen), or a combination of the two: These treatments are not without severe side effects. Paclitaxel inhibits microtubule disassembly and is active against a wide range of human tumors. The major side effects of paclitaxel are neutropenia, neurotoxicity, and cardiotoxicity. Doxorubicin is one of the most active cytotoxic agents against breast cancer, but side effects, such as cardiotoxicity and myelosuppression, have hindered this drug's use in adjuvant therapy. Tamoxifen is the only drug approved by the United States Food and Drug Administration for breast cancer risk reduction in estrogen-sensitive breast cancer. Women receiving tamoxifen may experience more frequent hot flashes, the development of cataracts, and increased risk for venous thromboembolic events and strokes. Tamoxifen use is also associated with

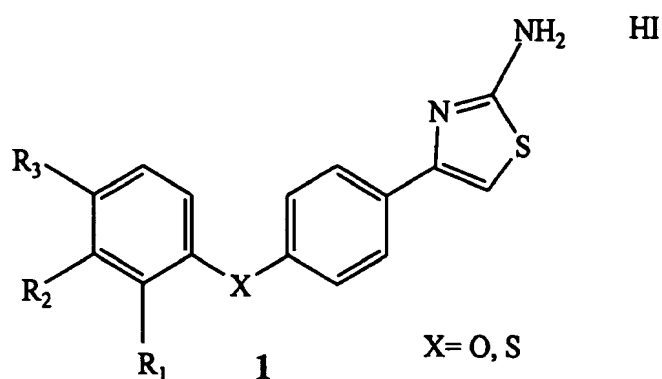
increased endometrial cancer risk in postmenopausal women with a uterus. As a result of the side effects caused by the current adjuvant treatments, there is a need for safer, more potent breast cancer agents.

2-Aminothiazoles represent a fairly new class of breast cancer drugs with only a few examples in the literature (Figure 1). Currently, a number of aminothiazolecarbonitriles<sup>6</sup> and thiazolylaminopyridines<sup>7,8</sup> are being investigated for their use as tyrosine kinase inhibitors. Aminothiazole inhibitors of cyclin-dependent kinase 2 have been shown to have significant antitumor activity in breast cancer models.<sup>9</sup> Thiophene-2 carboxamides containing 2-aminothiazoles have been evaluated as serine protease urokinase inhibitors.<sup>10</sup> In addition to 2-aminothiazoles, there are also few examples in the breast cancer literature of compounds containing diaryl ethers. A number of diaryl ether compounds have been evaluated as anti-cancer agents,<sup>11</sup> however, there are no reports of diaryl ethers with a 2-aminothiazole moiety. The present invention is directed to the synthesis of a number of 4-aryloxy- and 4-arylsulfanyl-phenyl-2-aminothiazoles (Figure 2), pharmaceutical compositions comprising such compounds and their use as anticancer agents. The compounds are evaluated for efficacy against a panel of human breast cancer cell lines that represent a clinical spectrum of estrogen positive, estrogen-negative, and adriamycin-resistant breast cancers.



**Fig. 1**

Examples of current 2-aminothiazoles being investigated for breast cancer activity



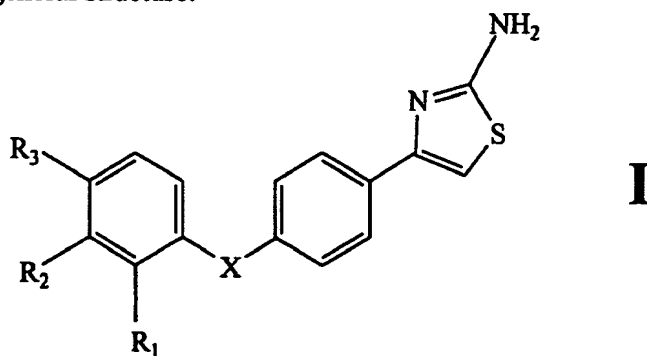
**Fig. 2**

General structure of 4-aryloxy- and 4-arylsulfanyl-phenyl-2-aminothiazole salts

### Detailed Description

Several substituted 4-aryloxy- and 4-arylsulfanyl-phenyl-2-aminothiazoles have been synthesized and evaluated for cytotoxic activity against estrogen-positive, estrogen negative, and adriamycin-resistant human breast cancer cell lines. 4-[4'-(3,4-Dichlorophenoxy)-phenyl]-thiazol-2-yl ammonium iodide demonstrated potent activity against both estrogen- positive and negative breast cancer cell lines with low micromolar ( $\mu\text{M}$ )  $\text{GI}_{50}$  values. In addition, we have identified several 2-aminothiazoles that demonstrated selective potency for the adriamycin-resistant and estrogen-negative breast cancer cell lines. The results suggest that these 2-aminothiazoles represent lead compounds for evaluation in animal models of breast cancer.

In accordance with the present invention an anticancer agent is provided wherein the agent has the general structure:



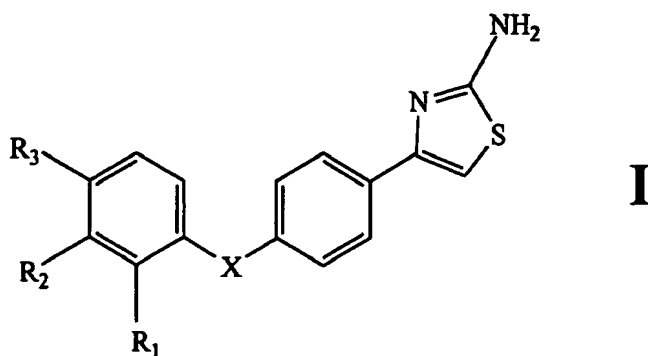
wherein X is selected from the group consisting of O, S and NH and  $\text{R}_1$ ,  $\text{R}_2$ , and  $\text{R}_3$  are independently selected from the group consisting of H, halo,  $(\text{C}_1\text{-C}_4)\text{alkyl}$ ,  $(\text{C}_1\text{-C}_4)\text{alkoxy}$ , aryl, -O-aryl and  $(\text{CO})\text{OR}_4$ , wherein  $\text{R}_4$  is H or  $(\text{C}_1\text{-C}_4)\text{alkyl}$ . In one embodiment, X is O or S

$R_1$  is H and  $R_2$ , and  $R_3$  are independently selected from the group consisting of H, halo,  $(C_1-C_4)$ alkyl,  $(C_1-C_4)$ alkoxy, phenyl, -O-phenyl and  $(CO)OR_4$ , wherein  $R_4$  is  $(C_1-C_4)$ alkyl. In another embodiment the compounds has the general structure of Formula I, wherein X is O or S,  $R_1$  is H and  $R_2$ , and  $R_3$  are independently selected from the group consisting of H, Cl,  $(C_1-C_2)$ alkyl,  $(C_1-C_2)$ alkoxy, phenyl, -O-phenyl and  $(CO)OCH_2CH_3$  and pharmaceutically acceptable salts thereof.

One embodiment of the present invention is directed to pharmaceutical compositions comprising the compounds of the invention and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier can be selected from among the group consisting of excipients, disintegrating agents, binders and lubricating agents. The amount of the pharmaceutical agent suitable for administration will be in accordance with standard clinical practice. In addition the pharmaceutical compositions can be further combined with other known anti-tumor agents and used in conjunction with known anti-tumor therapies.

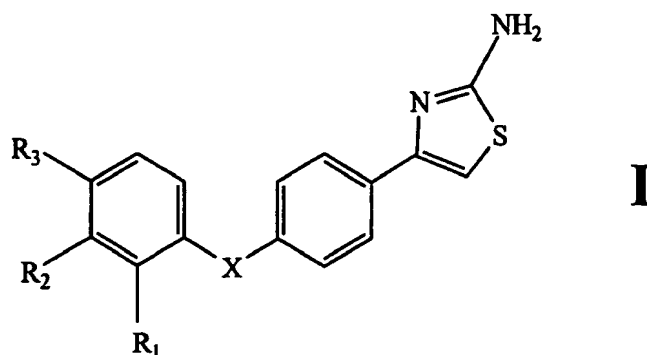
The anticancer compositions of the present invention can be administered either orally or parenterally. In one embodiment the composition is administered locally by injection or by an implantable time release device. When administered orally, the compounds can be administered as a liquid solution, powder, tablet, capsule or lozenge. The compounds can be used in combination with one or more conventional pharmaceutical additives or excipients used in the preparation of tablets, capsules, lozenges and other orally administrable forms. When administered parenterally, and more preferably by intravenous injection, the sodium channel blockers of the present invention can be admixed with saline solutions and/or conventional IV solutions.

In accordance with one embodiment of the present invention a method is provided for inhibiting the proliferation of neoplastic cells, and more particularly in one embodiment, breast cancer cells. The method comprises contacting the cells with a compound represented by the general structure:



wherein X is selected from the group consisting of O, S and NH and R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are independently selected from the group consisting of H, halo, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, aryl, -O-aryl and (CO)OR<sub>4</sub>, wherein R<sub>4</sub> is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl. In one embodiment, X is O or S, R<sub>1</sub> is H and R<sub>2</sub>, and R<sub>3</sub> are independently selected from the group consisting of H, halo, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, phenyl, -O-phenyl and (CO)OR<sub>4</sub>, wherein R<sub>4</sub> is (C<sub>1</sub>-C<sub>4</sub>)alkyl. In another embodiment the compounds has the general structure of Formula I, wherein X is O or S, R<sub>1</sub> is H and R<sub>2</sub>, and R<sub>3</sub> are independently selected from the group consisting of H, Cl, (C<sub>1</sub>-C<sub>2</sub>)alkyl, (C<sub>1</sub>-C<sub>2</sub>)alkoxy, phenyl, -O-phenyl and (CO)OCH<sub>2</sub>CH<sub>3</sub> and pharmaceutically acceptable salts thereof.

In accordance with one embodiment of the present invention a method is provided for treating a warm blooded vertebrate patient, including humans, afflicted by a neoplastic disease, such as breast cancer. The method comprises the steps of administering to such a patient an effective amount of a composition comprising a compound represented by the general structure:



wherein X is selected from the group consisting of O, S and NH and R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are independently selected from the group consisting of H, halo, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, aryl, -O-aryl and (CO)OR<sub>4</sub>, wherein R<sub>4</sub> is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl and pharmaceutically acceptable salts thereof. In one embodiment, X is O or S, R<sub>1</sub> is H and R<sub>2</sub>, and R<sub>3</sub> are independently selected from the group consisting of H, halo, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, phenyl, -O-phenyl and (CO)OR<sub>4</sub>, wherein R<sub>4</sub> is (C<sub>1</sub>-C<sub>4</sub>)alkyl. In another embodiment the compounds has the general structure of Formula I, wherein X is O or S, R<sub>1</sub> is H and R<sub>2</sub>, and R<sub>3</sub> are independently selected from the group consisting of H, Cl, (C<sub>1</sub>-C<sub>2</sub>)alkyl, (C<sub>1</sub>-C<sub>2</sub>)alkoxy, phenyl, -O-phenyl and (CO)OCH<sub>2</sub>CH<sub>3</sub> and pharmaceutically acceptable salts thereof. In one embodiment X is O, R<sub>1</sub> is H, R<sub>2</sub> is selected from the group consisting of H, Cl, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, phenyl, -O-phenyl, and (CO)OR<sub>4</sub>, and R<sub>3</sub> is selected from the group consisting of H, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, phenyl, -O-phenyl and (CO)OR<sub>4</sub>, wherein R<sub>4</sub> is (C<sub>1</sub>-C<sub>4</sub>)alkyl.

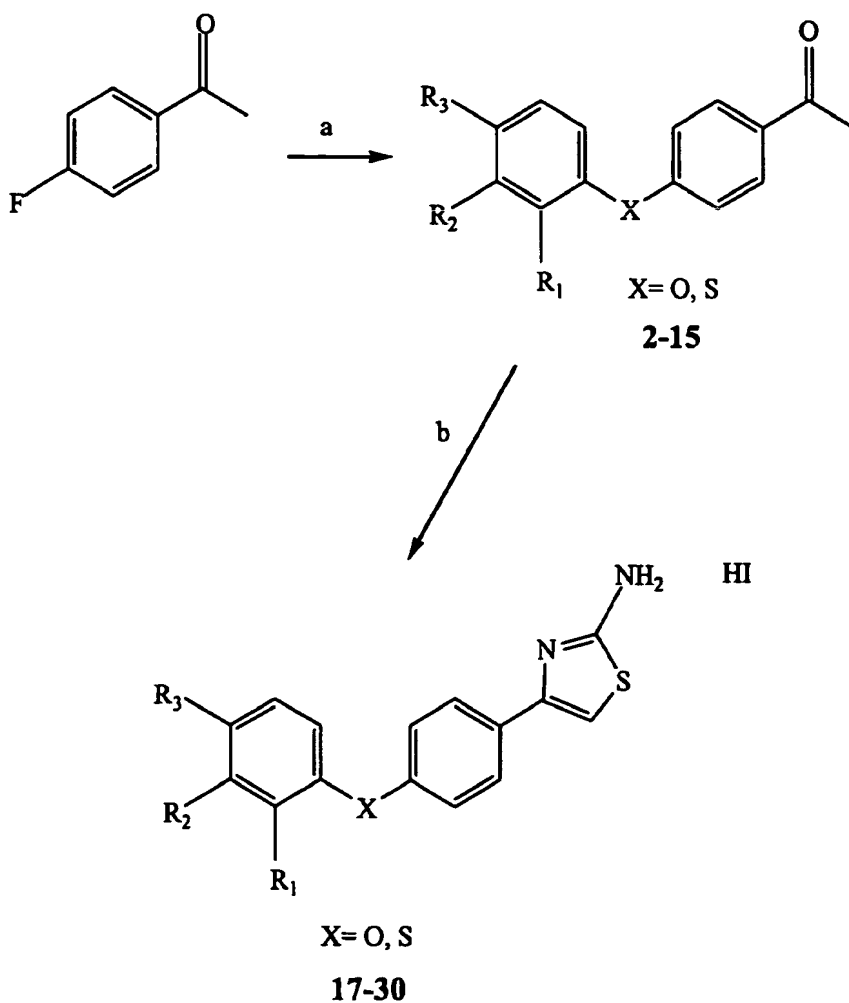
### Example 1

#### In vitro activity of the 4-aryloxy- and 4-arylsulfanyl-phenyl-2 aminothiazole compounds

#### Results and Discussion

A general synthesis of the substituted 4-aryloxy- and 4-arylsulfanyl-phenyl-2 aminothiazole salts is shown in Scheme 1.

Scheme 1



Reagents and conditions: a) phenol or thiol, K<sub>2</sub>CO<sub>3</sub>, DMAC, reflux, 8-10 h, 38-93%; b) thiourea, I<sub>2</sub>, EtOH, 100°C, 3 h, 40-98%

Condensation of the appropriately substituted phenols with 4'-fluoroacetophenone afforded the corresponding 4-aryloxyacetophenones in 40-93% yield.<sup>12</sup> This condensation reaction also



proceeded smoothly with various substituted benzenethiols forming the 4-arylsulfanylacetophenones in 38-87% yield. Treatment of the acetophenones with thiourea and iodine successfully generated the 2 aminothiazole salts (Table 1) in 40-98% yield. 13

Upon completion of the synthesis, thiazole compounds 17-24 and 27-30 were submitted to the NCI's antitumor screen and the results against human breast cancer cell lines are shown in Table 2 as GI<sub>50</sub> values. Analogues 16, 25, and 26 were evaluated for growth inhibition of MCF- 7 at 100  $\mu$ M. Compounds 20 and 28 showed selectivity for the adriamycin-resistant cell line with GI<sub>50</sub> values of 1.29  $\mu$ M and 3.37  $\mu$ M, respectively.

Adriamycin-resistant selectivity is clinically important because patients develop cancer that is resistant to treatment.

Interestingly, when the GI<sub>50</sub> values of compounds 17 (X=O) and 27 (X=S) are compared, there appears to be a relationship between heteroatom substitution and selectivity for the estrogen-positive or estrogen-negative cell lines. The GI<sub>50</sub> of 17 exceeded 100  $\mu$ M in all cell lines except for the estrogen-positive cell line T-47D in which the GI<sub>50</sub> was 0.917  $\mu$ M, while the GI<sub>50</sub> of 27 was 24.4  $\mu$ M for the T -47D cell line.

Compound 27 showed greater selectivity for the estrogen-negative cell line MDA-MB 435 with a GI<sub>50</sub> of 1.49  $\mu$ M when compared with greater than 100  $\mu$ M for 17. The aforementioned selectivity was also seen for 22 and 30 for the same cell lines. These results strongly suggest that estrogen-positive selectivity appears to be achieved using an oxygen linkage whereas compounds with a sulfur linkage are significantly more active against estrogen-negative breast cancer cell types. Thiazole 20 exhibited low micromolar cytotoxicity in all breast cancer cell lines except T-47D and was the only compound to show significant cytotoxicity in the estrogen-negative HS-578T cell line with a GI<sub>50</sub> of 2  $\mu$ M. Low micromolar cytotoxicity was also observed for 28 which was active in all cell lines except HS 578T with GI<sub>50</sub> values in the 1-5  $\mu$ M range. Compounds 20 and 28 have similar 3,4-dichloro substitutions on their outer phenyl ring and differ only in the heteroatom that links the two rings. Thiazoles 22-24 demonstrated selectivity for the estrogen-positive cell line T-47D with GI<sub>50</sub> values of 0.54  $\mu$ M, 3.91  $\mu$ M, and 4.90  $\mu$ M, respectively. Compound 30 was selective against the MDA-MB-435 cell line with a GI<sub>50</sub> of 1.4  $\mu$ M. In addition to the selectivity attained with different linkages between the two phenyl rings, these thiazoles also exhibit several distinct structure-activity relationships for substitution on the outer phenyl ring.

For the oxygen linker series, thiazole 22 with an electron-donating para-methyl substituent shows increased efficacy over the electron-withdrawing para-chloro thiazole 17 in all cell lines. An increase in bulk at the para position from methyl to phenyl does

not significantly alter activity. Chlorine substitution at the ortho, meta, and para positions was also investigated with compounds 17-19. In most of the cell lines, the best activity was achieved by chloro substitution at the meta position. Substitution at the ortho position showed a slight decrease in activity whereas the para-chloro thiazole had GI<sub>50</sub> values greater than 100  $\mu$ M in all cell lines except T-47D. Although the meta- and para-chloro thiazoles were less active than the ortho-chloro compound, chlorine substitution at both the meta and para positions provided the most active compound out of this series. While a para electron-donating group was more active (compared GI<sub>50</sub> of 22 to that of 17) than a para electron-withdrawing group for the oxygen linker series, the opposite relationship was observed for compounds with the thioether linkage. Generally, for this series of compounds, the para-chloro thiazole was more active (compared GI<sub>50</sub> of 27 to that of 30) than the para-methyl thiazole.

## Conclusion

In conclusion, we have synthesized a series of 4-aryloxy- and 4-arylsulfanyl-phenyl-2-aminothiazoles. Compounds 17, 18, 22, 24, 27, and 30 demonstrated selective cytotoxicity of either estrogen-positive or negative breast cancer cells while compounds 20 and 28 showed low micromolar growth inhibition of most human breast tumor cells. In general, thiazoles with an oxygen linkage showed estrogen-positive selectivity whereas estrogen-negative selectivity was achieved by thioether linkages. Thiazoles 20 and 28, both with 3,4-dichloro substitutions, exhibited selectivity for the adriamycin resistant cell line. These compounds have the potential to be promising lead compounds for mechanistic studies on breast cancer cell cycle arrest and for the further development of selective thiazole-containing breast cancer agents.

## Experimental Section

All starting materials were purchased from Aldrich and were used as received. Melting points were measured on an Electrothermal Mel-Temp and are uncorrected. Carbon and proton NMR spectra were recorded on a General Electric 300 MHz spectrometer. Mass spectra were obtained on a Finnigan LcQ Classic spectrometer. High resolution (EI) mass spectra were obtained from the University of Illinois, Urbana-Champaign. Combustion analyses were performed by Atlantic Microlabs Inc.

**General Procedure for Synthesis of 4-Aryloxy and 4-Arylsulfanyl Substituted Acetophenones<sup>12</sup> (2-15).** Anhydrous K<sub>2</sub>CO<sub>3</sub> (12 mmol) was added to a solution of 4'-

fluoroacetophenone (10 mmol) and the corresponding phenol or thiol (10 mmol) in N,N-dimethylacetamide (DMAC, 10 mL). The suspension was refluxed for 8-10 h, cooled to room temperature, and diluted with H<sub>2</sub>O (10 mL). In some instances (**8** and **9**), the addition of H<sub>2</sub>O resulted in the deposition of the product as a solid which was collected by filtration. In those instances where the product was not a solid, the resulting solution was extracted with CHCl<sub>3</sub> (3 × 15 mL), dried over MgSO<sub>4</sub>, and concentrated *in vacuo* to yield a brown oil. The remaining DMAC was removed by Kugelrohr distillation. The viscous oil was allowed to cool and solidify. The crude solid was recrystallized from EtOH.

**4-(4'-Chlorophenoxy)-acetophenone (2).** Isolated as a light brown solid (1.81 g, 73%); mp 65-66 °C (lit.<sup>12</sup> 66-68 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.57 (s, 3H), 6.99 (d, *J* = 6.4 Hz, 4H), 7.35 (d, *J* = 8.3 Hz, 2 H), 7.94 (d, *J* = 8.2 Hz, 2H); <sup>13</sup>C NMR δ 27.0, 117.8, 121.7, 130.2, 130.6, 131.2, 132.8, 154.7, 162.0, 197.1; MS APCI *m/z* 247 (M)<sup>+</sup>.

**4-(3'-Chlorophenoxy)-acetophenone (3).** Isolated as an orange oil (2.03 g, 82%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.49 (s, 3H), 6.82-7.13 (m, 5H), 7.22 (t, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 2H); <sup>13</sup>C NMR δ 26.9, 109.9, 118.3, 118.5, 120.6, 125.0, 131.1, 133.0, 135.7, 157.0, 161.4, 196.9; MS APCI *m/z* 247 (M)<sup>+</sup>.

**4-(2'-Chlorophenoxy)-acetophenone (4).** Isolated as a yellow solid (1.64 g, 67%); mp 41-43 °C (lit.<sup>16</sup> 49-50 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.47 (s, 3H), 6.85 (d, *J* = 8.3 Hz, 2H), 6.99-7.16 (m, 2H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 7.5 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 2H); <sup>13</sup>C NMR δ 26.9, 109.9, 116.8, 123.0, 126.7, 127.2, 128.9, 131.1, 131.5, 132.5, 151.2, 161.7, 196.9; MS APCI *m/z* 247 (M)<sup>+</sup>.

**4-(3',4'-Dichlorophenoxy)-acetophenone (5).** Recrystallized from hexanes to afford a brown solid (2.61, 93%); mp 47-49 °C (lit.<sup>17</sup> 50-52 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.57 (s, 3H), 6.90 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 7.14 (s, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 2H); <sup>13</sup>C NMR δ 27.0, 118.4, 119.7, 122.2, 128.5, 131.2, 131.8, 133.3, 134.0, 155.3, 161.2, 197.1; MS APCI *m/z* 281 (M)<sup>+</sup>.

**4-(4'-Methoxyphenoxy)-acetophenone (6).** Isolated as a light brown solid (2.02 g, 84%); mp 56-58 °C (lit.<sup>18</sup> 61°C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.55 (s, 3H), 3.81 (s, 3H), 6.90-7.00 (m, 6H), 7.90 (d, *J* = 7.9 Hz, 2H); <sup>13</sup>C NMR δ 26.9, 56.1, 115.6, 116.9, 122.2, 131.1, 131.9, 149.0, 157.2, 163.4, 197.2; MS APCI *m/z* 243 (M)<sup>+</sup>.

**4-(*p*-Toluoxy)-acetophenone (7).** Isolated as a light brown solid (0.91 g, 40%); mp 44-46 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.37 (s, 3H), 2.57 (s, 3H), 6.97 (d, *J* = 7.5 Hz, 4H), 7.19 (d, *J* = 7.7 Hz, 2H), 7.92 (d, *J* = 8.5 Hz, 2H); <sup>13</sup>C NMR δ 21.3, 26.9, 117.4, 120.5, 120.7, 131.1, 132.1, 134.9, 153.5, 163.0, 197.2; MS APCI *m/z* 227 (M)<sup>+</sup>.

**4-(Biphenyl-4'-yloxy)-acetophenone (8).** Isolated as a pale yellow solid (2.61 g, 90%); mp 114-117 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.59 (s, 3H), 7.06 (d, *J* = 8.3 Hz, 2H), 7.14 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 7.1 Hz, 4H), 7.96 (d, *J* = 8.0 Hz, 2H); <sup>13</sup>C NMR δ 27.0, 117.9, 120.9, 127.5, 127.8, 129.2, 129.4, 131.1, 132.5, 138.2, 140.8, 155.5, 162.4, 197.2; MS APCI *m/z* 289 (M)<sup>+</sup>.

**4-(4'-Phenoxy-phenoxy)-acetophenone (9).** Isolated as a white solid (2.47 g, 81%); mp 75-77 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.57 (s, 3H), 6.80-7.40 (m, 11H), 7.94 (d, *J* = 8.1 Hz, 2H); <sup>13</sup>C NMR δ 26.9, 117.3, 119.2, 120.9, 122.1, 123.9, 130.3, 131.1, 132.3, 151.3, 154.5, 157.8, 162.9, 197.2; MS APCI *m/z* 305 (M)<sup>+</sup>.

**Ethyl 3-(4'-acetyl-phenoxy)-benzoate (10).** Isolated as a yellow oil (1.92 g, 68%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.37 (t,  $J = 6.7$  Hz, 3H), 2.56 (s, 3H), 4.35 (q,  $J = 7.1$  Hz, 3H), 6.98 (d,  $J = 8.1$  Hz, 2H), 7.24 (d,  $J = 7.9$  Hz, 1H), 7.44 (t,  $J = 7.5$  Hz, 1H), 7.71 (s, 1H), 7.86 (d,  $J = 7.5$  Hz, 1H), 7.93 (d,  $J = 8.1$  Hz, 3H);  $^{13}\text{C}$  NMR  $\delta$  14.8, 26.9, 61.8, 118.0, 121.5, 125.0, 126.1, 130.5, 131.2, 132.8, 133.2, 156.1, 161.9, 166.2, 197.1; MS APCI  $m/z$  285 ( $\text{M}$ ) $^+$ .

**(4'-Phenylsulfanyl)-acetophenone (11).** Isolated as an orange solid (0.87 g, 38%); mp 59-61  $^\circ\text{C}$  (lit.<sup>19</sup> 67-68  $^\circ\text{C}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.55 (s, 3H), 7.21 (d,  $J = 8.1$  Hz, 2H), 7.40-7.47 (m, 5H), 7.82 (d,  $J = 8.1$  Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  13.7, 128.0, 129.3, 129.4, 130.2, 132.6, 134.4, 135.0, 145.4, 192.9; MS APCI  $m/z$  229 ( $\text{M}$ ) $^+$ .

**4-(4'-Chloro-phenylsulfanyl)-acetophenone (12).** Isolated as an orange solid (1.68 g, 64%); mp 40-42  $^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.56 (s, 3H), 7.22 (d,  $J = 8.1$  Hz, 2H), 7.38 (d,  $J = 6.5$  Hz, 4H), 7.83 (d,  $J = 7.9$  Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  27.0, 128.3, 129.5, 130.4, 131.4, 135.4, 143.7, 144.5, 147.8, 197.5; MS APCI  $m/z$  263 ( $\text{M}$ ) $^+$ .

**4-(3',4'-Dichloro-phenylsulfanyl)-acetophenone (13).** Isolated as a red oil (1.44 g, 87%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.56 (s, 3H), 7.23-7.33 (m, 2H), 7.39-7.53 (m, 1H), 7.85 (d,  $J = 8.1$  Hz, 4H);  $^{13}\text{C}$  NMR  $\delta$  27.0, 129.4, 129.7, 131.6, 131.8, 132.3, 133.6, 133.9, 134.0, 134.5, 136.0, 197.5; MS APCI  $m/z$  297 ( $\text{M}$ ) $^+$ .

**4-(4'-Methoxy-phenylsulfanyl)-acetophenone (14).** Isolated as a red solid (1.49 g, 58%); mp 25-27  $^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.51 (s, 3H), 3.83 (s, 3H), 6.94 (d,  $J = 8.1$  Hz, 2H), 7.07 (d,  $J = 8.1$  Hz, 2H), 7.45 (d,  $J = 8.1$  Hz, 2H), 7.76 (d,  $J = 8.1$  Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  26.9, 55.9, 115.9, 121.8, 126.3, 129.3, 134.4, 137.3, 147.4, 161.2, 197.6; MS APCI  $m/z$  259 ( $\text{M}$ ) $^+$ .

**4-(4'-Tolylsulfanyl)-acetophenone (15).** Isolated as a red solid (1.64 g, 68%); mp 90-92 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.40 (s, 3H), 2.54 (s, 3H), 7.15 (d,  $J$  = 8.1 Hz, 2H), 7.22 (d,  $J$  = 7.7 Hz, 2H), 7.41 (d,  $J$  = 7.5 Hz, 2H), 7.79 (d,  $J$  = 8.0 Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  21.8, 27.0, 127.2, 128.4, 129.3, 131.0, 134.6, 135.0, 140.0, 146.4, 197.6; MS APCI  $m/z$  243 ( $\text{M}$ ) $^+$ .

**General Procedure for Synthesis of Substituted 4-Aryloxy and 4-Arylsulfanyl-phenyl-2-aminothiazole salts<sup>13</sup> (16-30).**

Thiourea (40 mmol) and iodine (11 mmol) were added to a stirring solution of the appropriate acetophenone (10 mmol) in absolute ethanol (20 mL). The mixture was heated at 100 °C for 2-3 h in an open vessel. The crude residue was washed with ether (3  $\times$  50 mL) and was recrystallized from hot water. A few of these compounds ( $16^{20}$ ,  $17^{21}$ , and  $26^{20}$ ) in free amine or HCl salt form are reported in the literature.

**4-(4'-Phenoxyphenyl)-thiazol-2-yl ammonium iodide (16).** Isolated as a yellow solid (1.82 g, 46%); mp 193-195 °C;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  7.02 (t,  $J$  = 8 Hz, 4H), 7.08 (s, 1H), 7.13 (t,  $J$  = 6.2 Hz, 1H), 7.37 (t,  $J$  = 7.7 Hz, 2H), 7.65 (d,  $J$  = 8.1 Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  103.0, 119.5, 120.1, 125.1, 128.8, 131.2, 140.1, 145.7, 156.4, 158.6, 171.1; MS APCI  $m/z$  269 ( $\text{M-HI}$ ) $^+$ ; Anal. calcd. for  $\text{C}_{15}\text{H}_{13}\text{IN}_2\text{OS}$ : C, 45.47; H, 3.31; N, 7.07. Found: C, 45.25; H, 3.26; N, 7.08.

**4-[4'-(4-Chlorophenoxy)-phenyl]-thiazol-2-yl ammonium iodide (17).** Isolated as a light orange solid (0.31 g, 71%); mp 152-154 °C;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  6.95-7.10 (m, 5H), 7.41 (t,  $J$  = 6.6 Hz, 2H), 7.67 (d,  $J$  = 8.7 Hz, 1H), 7.92 (d,  $J$  = 8.7 Hz, 1H), 8.94 (br s);  $^{13}\text{C}$  NMR  $\delta$  109.8, 118.6, 118.8, 121.9, 123.1, 128.9, 131.0, 131.2, 131.7, 155.6, 170.0; HRMS calcd for  $\text{C}_{15}\text{H}_{12}\text{ClIN}_2\text{OS}$  ( $\text{M-HI}$ ) $^+$   $m/z$  303.036100, found  $m/z$  303.035888.

**4-[4-(3'-Chlorophenoxy)-phenyl]-thiazol-2-yl ammonium iodide (18).** Isolated as a yellow solid (0.57 g, 65%); mp 107-110 °C; <sup>1</sup>H NMR (DMSO-d<sup>6</sup>) δ 6.96 (d, *J* = 7.9 Hz, 1H), 7.11 (s, 1H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 4H), 7.93 (d, *J* = 7.8 Hz, 1H), 8.96 (br s); <sup>13</sup>C NMR δ 103.2, 113.9, 118.4, 119.7, 120.2, 124.8, 128.9, 132.6, 135.0, 144.1, 157.6, 160.6, 171.0; HRMS calcd for C<sub>15</sub>H<sub>12</sub>ClIN<sub>2</sub>OS (M-HI)<sup>+</sup> *m/z* 303.036700, found *m/z* 303.035888; Anal. calcd. for C<sub>15</sub>H<sub>12</sub>ClIN<sub>2</sub>OS·0.6 H<sub>2</sub>O: C, 40.81; H, 2.72; N, 6.34. Found: C, 40.53; H, 2.60; N, 6.10.

**4-[4-(2'-Chlorophenoxy)-phenyl]-thiazol-2-yl ammonium iodide (19).** Isolated as a yellow solid (0.35 g, 40%); mp 160-163 °C; <sup>1</sup>H NMR (DMSO-d<sup>6</sup>) δ 6.98 (d, *J* = 8.3 Hz, 2H), 7.08 (s, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 7.23 (t, *J* = 6.6 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 2H), 8.99 (br s); <sup>13</sup>C NMR δ 103.0, 118.2, 123.0, 126.9, 127.2, 128.9, 130.0, 131.7, 131.8, 133.4, 147.5, 158.3, 171.0; MS APCI *m/z* 303 (M-HI)<sup>+</sup>; Anal. calcd. for C<sub>15</sub>H<sub>12</sub>ClIN<sub>2</sub>OS: C, 41.83; H, 2.81; N, 6.50. Found: C, 41.65; H, 2.71; N, 6.48.

**4-[4'-(3,4-Dichlorophenoxy)-phenyl]-thiazol-2-yl ammonium iodide (20).** Isolated as a brown solid (0.35 g, 75%); mp 202-205 °C; <sup>1</sup>H NMR (DMSO-d<sup>6</sup>) δ 7.07 (d, *J* = 7.5 Hz, 3H), 7.14 (s, 1H), 7.37 (s, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 9.02 (br s); <sup>13</sup>C NMR δ 103.4, 118.9, 120.2, 120.9, 121.7, 122.6, 129.0, 131.7, 132.7, 133.2, 133.5, 161.0, 171.1; HRMS calcd for C<sub>15</sub>H<sub>11</sub>Cl<sub>2</sub>IN<sub>2</sub>OS (M-HI)<sup>+</sup> *m/z* 336.997000, found *m/z* 336.996915; Anal. calcd. for C<sub>15</sub>H<sub>11</sub>Cl<sub>2</sub>IN<sub>2</sub>OS·0.9 H<sub>2</sub>O: C, 37.43; H, 2.28; N, 5.82. Found: C, 37.19; H, 2.25; N, 5.66.

**4-[4'-(4-Methoxyphenoxy)-phenyl]-thiazol-2-yl ammonium iodide (21).** Isolated as a yellow solid (0.37 g, 87%); mp 204-207 °C; <sup>1</sup>H NMR (DMSO-d<sup>6</sup>) δ 3.71 (s, 3H), 6.95-

7.03 (m, 5H), 7.05 (s, 1H), 7.62 (d,  $J = 8.2$  Hz, 2H), 7.98 (d,  $J = 7.9$  Hz, 1H), 8.93 (br s);  $^{13}\text{C}$  NMR  $\delta$  56.4, 102.6, 116.2, 117.1, 118.2, 122.1, 122.6, 124.5, 128.7, 131.6, 150.0, 171.1; MS APCI  $m/z$  299 (M-HI) $^{+}$ ; Anal. calcd. for  $\text{C}_{16}\text{H}_{15}\text{IN}_2\text{O}_2\text{S}$ : C, 45.08; H, 3.55; N, 6.57. Found: C, 44.97; H, 3.65; N, 6.67.

**4-[4'-(*p*-Toluoxy)phenyl]-thiazol-2-yl ammonium iodide (22).** Isolated as a light brown solid (0.27 g, 65%); mp 118-120  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  2.26 (s, 3H), 6.90-7.03 (m, 5H), 7.19 (t,  $J = 7.9$  Hz, 2H), 7.65 (d,  $J = 8.6$  Hz, 1H), 7.90 (d,  $J = 6.7$  Hz, 1H);  $^{13}\text{C}$  NMR  $\delta$  27.5, 117.6, 118.9, 120.3, 121.0, 128.6, 131.5, 131.6, 134.2, 134.9, 161.2, 162.6; MS APCI  $m/z$  283 (M-HI) $^{+}$ ; Anal. calcd. for  $\text{C}_{16}\text{H}_{15}\text{IN}_2\text{OS}$ : C, 46.84; H, 3.69, N, 6.83. Found: C, 46.65; H, 3.86; N, 6.61.

**4-[4'-(Biphenyl-4-yloxy)-phenyl]-thiazol-2-yl ammonium iodide (23).** Isolated as a yellow solid (0.33 g, 70%); mp 253-254  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  7.00-7.20 (m, 4H), 7.30 (d,  $J = 6.5$  Hz, 1H), 7.40 (t,  $J = 7.3$  Hz, 2H), 7.66 (m, 6H), 7.93 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C}$  NMR  $\delta$  102.8, 119.8, 120.4, 127.4, 128.2, 128.8, 129.4, 129.5, 129.9, 136.7, 137.3, 156.4, 158.2, 159.4, 177.5; HRMS calcd for  $\text{C}_{21}\text{H}_{17}\text{IN}_2\text{OS}$  (M-HI) $^{+}$   $m/z$  345.106200, found  $m/z$  345.106160; Anal. calcd. for  $\text{C}_{21}\text{H}_{17}\text{IN}_2\text{OS}$ : C, 53.40; H, 3.63; N, 5.93. Found. C, 53.65; H, 3.65; N, 5.80.

**4-[4'-(4-Phenoxy-phenoxy)-phenyl]-thiazol-2-yl ammonium iodide (24).** Isolated as a pale yellow solid (0.42 g, 86%); mp 137-140  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$ )  $\delta$  6.95-7.12 (m, 10H), 7.34 (t,  $J = 7.4$  Hz, 2H), 7.67 (d,  $J = 8.1$  Hz, 1H), 7.92 (d,  $J = 8.3$  Hz, 1H);  $^{13}\text{C}$  NMR  $\delta$  102.8, 117.7, 119.2, 119.3, 121.4, 122.0, 122.7, 124.3, 128.8, 131.0, 131.7, 143.9, 152.3, 153.3, 161.1; HRMS calcd for  $\text{C}_{21}\text{H}_{17}\text{IN}_2\text{O}_2\text{S}$  (M-HI) $^{+}$   $m/z$  361.101000, found  $m/z$  361.101075.



**4-[4-(3'-Ethoxycarbonyl-phenoxy)-phenyl]-thiazol-2-yl ammonium iodide (25).**

Isolated as a yellow solid (1.01 g, 64%); mp 158-160 °C;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  1.24 (t,  $J = 6.3$  Hz, 3H), 4.24 (q,  $J = 7.0$  Hz, 2H), 7.10 (s, 3H), 7.34 (d,  $J = 7.9$  Hz, 1H), 7.45 (s, 1H), 7.51 (t,  $J = 7.7$  Hz, 1H), 7.71 (d,  $J = 7.2$  Hz, 2H), 7.94 (d,  $J = 7.9$  Hz, 1H), 8.83 (br s);  $^{13}\text{C}$  NMR  $\delta$  15.0, 62.0, 103.2, 109.8, 118.7, 119.6, 120.1, 124.8, 125.5, 129.0, 131.8, 132.8, 157.3, 157.9, 165.9, 171.0; HRMS calcd for  $\text{C}_{18}\text{H}_{17}\text{IN}_2\text{O}_3\text{S}$  (M-HI) $^+$   $m/z$  341.095700, found  $m/z$  341.095989; Anal. calcd. for  $\text{C}_{18}\text{H}_{17}\text{IN}_2\text{O}_3\text{S} \cdot 2 \text{H}_2\text{O}$ : C, 42.87; H, 3.37; N, 5.55. Found: C, 42.58; H, 3.26; N, 5.51.

**4-(4'-Phenylsulfanyl-phenyl)-thiazol-2-yl ammonium iodide (26).** Isolated as an orange solid (0.40 g, 98%); mp 129-132 °C;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.15 (s, 1H), 7.26-7.47 (m, 6H), 7.65 (d,  $J = 7.5$  Hz, 2H), 7.81 (d,  $J = 7.5$  Hz, 1H);  $^{13}\text{C}$  NMR  $\delta$  104.0, 127.7, 128.4, 129.0, 130.0, 130.7, 131.1, 132.5, 164.0, 170.8, 177.5; HRMS calcd for  $\text{C}_{15}\text{H}_{13}\text{IN}_2\text{S}_2$  (M-HI) $^+$   $m/z$  285.052200, found  $m/z$  285.052017.

**4-[4-(4'-Chloro-phenylsulfanyl)-phenyl]-thiazol-2-yl ammonium iodide (27).** Isolated as an orange solid (0.44 g, 98%); mp 158-160 °C;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.14 (s, 1H), 7.24-7.47 (m, 6H), 7.68 (d,  $J = 7.9$  Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  101.3, 110.1, 127.6, 127.9, 130.6, 132.0, 133.5, 143.6, 154.7, 167.8, 169.8; HRMS calcd for  $\text{C}_{15}\text{H}_{12}\text{ClIN}_2\text{S}_2$  (M-HI) $^+$   $m/z$  319.012900, found  $m/z$  319.013045.

**4-[4-(3',4'-Dichloro-phenylsulfanyl)-phenyl]-thiazol-2-yl ammonium iodide (28).**

Isolated as a red solid (0.23 g, 48%); mp 45-47 °C (dec.);  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  7.18 (s, 1H), 7.24 (s, 2H), 7.46 (d,  $J = 8.1$  Hz, 2H), 7.59 (s, 1H), 7.69 (d,  $J = 7.7$  Hz, 2H);  $^{13}\text{C}$  NMR  $\delta$  104.8, 128.1, 131.2, 131.3, 132.2, 132.6, 132.8, 133.0, 133.2, 140.1, 150.3, 161.2, 171.1; HRMS calcd for  $\text{C}_{15}\text{H}_{11}\text{Cl}_2\text{IN}_2\text{S}_2$  (M-HI) $^+$   $m/z$  352.974100, found  $m/z$  352.974073.

**4-[4-(4'-Methoxy-phenylsulfanyl)-phenyl]-thiazol-2-yl ammonium iodide (29).**

Isolated as an orange solid (0.40 g, 91%); mp 202-205 °C; <sup>1</sup>H NMR (DMSO-d<sup>6</sup>) δ 3.74 (s, 3H), 6.98 (d, *J* = 8.3 Hz, 2H), 7.07 (s, 1H), 7.12 (d, *J* = 7.9 Hz, 2H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 7.7 Hz, 2H); <sup>13</sup>C NMR δ 56.3, 103.4, 116.5, 116.7, 127.5, 128.4, 136.7, 140.3, 148.9, 154.3, 158.7, 169.7; MS APCI *m/z* 315 (M-HI)<sup>+</sup>; Anal. calcd. for C<sub>16</sub>H<sub>15</sub>IN<sub>2</sub>OS<sub>2</sub>: C, 43.44; H, 3.42; N, 6.33. Found: C, 43.18; H, 3.40; N, 6.32.

**4-(4'-*p*-Tolylsulfanyl)-phenyl)-thiazol-2-yl ammonium iodide (30).**

Isolated as an orange solid (0.23 g, 54%); mp 182-185 °C; <sup>1</sup>H NMR (DMSO-d<sup>6</sup>) δ 2.28 (s, 3H), 7.08 (s, 1H), 7.20 (m, 2H), 7.28 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.9 Hz, 4H); <sup>13</sup>C NMR δ 21.2, 109.9, 127.6, 128.7, 129.2, 130.0, 131.4, 133.4, 140.5, 153.8, 156.5, 170.5; MS APCI *m/z* 299 (M-HI)<sup>+</sup>; Anal. calcd. for C<sub>16</sub>H<sub>15</sub>IN<sub>2</sub>S<sub>2</sub>: C, 45.07; H, 3.55; N, 6.57. Found: C, 44.86; H, 3.52; N, 6.23.

**NCI High Throughput PreScreen.** Each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for 48 h. End-point determinations were made with alamar blue.<sup>15</sup> Results for each test agent were reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduce the growth of any one of the cell lines to approximately 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

**NCI Anti-tumor Screen.** Compounds were tested against 60 human tumor cell lines at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous drug exposure protocol<sup>22</sup> was used, and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth.

**Inhibition of Breast Cell Proliferation.** MCF7 cells were treated with compounds or vehicle (DMSO). After a 48 h incubation, the SRB assay was used to determine inhibition of proliferation and cytotoxicity.<sup>23</sup> Percent inhibition of growth at 100  $\mu$ M was determined and GI<sub>50</sub> values were calculated from log-dose response curves as previously described.<sup>24</sup>

### Acknowledgements

We thank the National Cancer Institute's Developmental Therapeutics Program for testing the thiazoles, the Leukemia and Lymphoma Society for funding to MJG, the William Randolph Hearst Foundation for funding to SLM, the University of Virginia Department of Chemistry, and the Burger Center of Biological Sciences for support.

### References

1. Ries, L.A.G.; Eisner, M.P.; Kosary, C.L.; Hankey, B.F.; Miller, B.A.; Clegg, L.; Mariotto, A.; Fay, M.P.; Feuer, E.J.; Edwards, B.K. (eds). *SEER Cancer Statistics Review*, 1973-2000, National Cancer Institute. Bethesda, MD, [http://seer.cancer.gov/csr/1975\\_2000](http://seer.cancer.gov/csr/1975_2000), 2003.
2. Alloatti, G.; Penna, C.; Gallo, M.P.; Levi, R.C.; Bombardelli, E.; Appendino, G. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 561-567.
3. Hortobagyi, G.N.; Buzdar, A.U. *Drugs*. **1993**, *45*, Suppl. 2:10-19.
4. Kaklaman, V.G.; Gradishar, W.J. *Clin. Breast Cancer* **2003**, *4*, Suppl. 1: S26-33.
5. Chlebowski, R.T.; Col, N.; Winer, E.P.; Collyar, D.E.; Cummings, S.R.; Vogel, V.G. III, Burstein, H.J.; Eisen, A.; Lipkus, I.; Pfister, D.G. *J. Clin. Oncol.* **2002**, *20*, 15, 3328-3343.
6. Bilodeau, M.T.; Manley, P.J.; Hartman, G.D. U.S. Patent 6,586,424, 2003.

7. Bilodeau, M.T.; Hartman, G.D. US Pat. Appl. 20030100567, 2003.
8. Bilodeau, M.T.; Hartman, G.D.; Hoffman, J.M.; Lumma, W.C., Jr.; Manley, P.J.; Rodman, L.; Sisko, J.T.; Smith, A.M.; Tucker, T.J. US Pat. Appl. 20020137755, 2002.
9. Kim, K.S.; Kimball, S.D.; Misra, R.N.; Rawlins, D.B.; Hunt, J.T.; Xiao, H.; Lu, S.; Qian, L.; Han, W.; Shan, W.; Mitt, T.; Cai, Z.; Poss, M.A.; Zhu, H.; Sack, J.S.; Tokarski, J.S.; Chang, C.Y.; Pavletich, N.; Kamath, A.; Humphreys, W.G.; Marathe, P.; Bursuker, I.; Kellar, O.K.A.; Roongta, U.; Batorsky, R.; Mulheron, J.G.; Bol, D.; Fairchild, C.R.; Lee, F.Y.; Webster, K.R. *J. Med. Chem.* **2002**, *45*, 18, 3905-3927.
10. Wilson, K.J.; Illig, C.R.; Subasinghe, N.; Hoffman, J.B.; Rudolph, M.J.; Soll, R.; Molloy, C.J.; Bone, R.; Green, D.; Randall, T.; Zhang, M.; Lewandowski, F.A.; Zhou, Z.; Sharp, C.; Maguire, D.; Grasberger, B.; DesJarlais, R.L.; Spurlino, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 7, 915-918.
11. Tuse, D.; Chen, X.; Laderoute, K.R.; Hiebert, C.K.; Olsen, C.M. U.S. Patent 6,169,104, 2001.
12. Yeager, G.W.; Schissel, D.N. *Synthesis*, **1991**, *1*, 63-68.
13. Chordia, M.D.; Murphree, L.J.; Macdonald, T.L.; Linden, J.; Olsson, R.A. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 12, 1563-1566.
14. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.*, **1991**, *83*, 757-766.
15. Gray, G.D.; Wickstrom, E. *Biotechniques*, **1996**, *21*, 5, 780-782.
16. Kimoto, S.; Asaki, K.; Kishi, S. *J. Pharm. Soc. Japan*, **1954**, *74*, 358-360; Chem. Abs. **1955**, 5373.

17. Markley, L.D.; Tong, Y.C.; Dulworth, J.K.; Steward, D.L.; Goralski, C.T.; Johnston, H.; Wood, S.G.; Vinogradoff, A.P.; Bargar, T.M. *J. Med. Chem.*, **1986**, *29*, 3, 427-433.
18. Petit, L.; Buu-Hoi, A. *J. Org. Chem.*, **1961**, *26*, 3832-3834.
19. Szmant, H.H.; Palopoli, F.P. *J. Amer. Chem. Soc.*, **1950**, *72*, 1757-1758.
20. Hargrave, K.D.; Hess, F.K.; Oliver, J.T. *J. Med. Chem.*, **1983**, *26*, 8, 1158-1163.
21. Kawamatsu, Y.; Sohda, T.; Imai, Y. *Eur. J. Med. Chem. Chim. Ther.*, **1981**, *16*, 4, 355-362.
22. For additional details concerning this screen and the NCI's drug discovery and development program, visit <http://dtp.nci.nih.gov>.
23. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S.; Boyd, M.R. *J. Natl. Cancer Ins.*, **1990**, *82*, 1107-1112.
24. Tinley, T.L.; Randall-Hlubek, D.A.; Leal, R.M.; Jackson, E.M.; Cessac, J.W.; Quada, J.C., Jr.; Hemscheidt, T.K.; Mooberry, S.L. *Cancer Res.*, **2003**, *63*, 3211-3220.

## Tables

**Table 1. Thiazoles**

Cmpd #	X	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	% Yield
17	O	H	H	Cl	71
18	O	H	Cl	H	65
19	O	Cl	H	H	40
20	O	H	Cl	Cl	75
21	O	H	H	OMe	87
22	O	H	H	Me	65
23	O	H	H	Ph	70
24	O	H	H	OPh	86
25	O	H	CO <sub>2</sub> Et	H	64
26	S	H	H	H	98
27	S	H	H	Cl	98
28	S	H	Cl	Cl	48
29	S	H	H	OMe	91
30	S	H	H	Me	54

**Table 2. Human Breast Cancer Cytotoxicity Data.**

Cmpd #	% Inhibition		NCI/ADR-RES	GI <sub>50</sub> (μM)				
	of MCF7 at 100 μM	MCF7		MDA-MB-231/ATCC	HS 578T	MDA-MB-435	BT-549	T-47D
16	81	ND	ND	ND	ND	ND	ND	ND
17	85	>100	>100	>100	>100	>100	>100	0.917
18	100	14.6	13.2	4.80	19.7	2.10	14.3	14.0
19	87	27.4	25.8	10.5	ND	14.8	12.8	16.4
20	47	3.02	1.29	2.29	2.00	0.759	1.91	20.9
21	24	ND	ND	ND	ND	ND	ND	ND
22	98	17.2	18.2	14.1	22.7	11.8	ND	0.54
23	53	14.3	17.4	12.6	18.8	18.6	10.4	3.91
24	81	35.1	35.9	12.6	27.8	22.7	16.1	4.9
25	84	ND	ND	ND	ND	ND	ND	ND
26	79	ND	ND	ND	ND	ND	ND	ND
27	89	21.6	17.5	12.4	18.8	1.49	15.3	24.4
28	54	5.68	3.37	3.73	ND	1.11	5.95	2.79
29	11	ND	ND	ND	ND	ND	ND	ND
30	96	25.9	64.6	>100	59.6	1.4	25.7	68

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/035586

International filing date: 27 October 2004 (27.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/514,678  
Filing date: 27 October 2003 (27.10.2003)

Date of receipt at the International Bureau: 24 November 2004 (24.11.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse